

Interaction effects of desiccation and temperature stress resistance across *Spodoptera frugiperda* (Lepidoptera, Noctuidae) developmental stages

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Abstract

Insects encounter multiple overlapping physiologically challenging environmental stressors in their habitats. As such, the ability of insects to withstand these stressors singly or interactively is fundamental in population persistence. Following its invasion in Africa, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) has successfully established and spread in most parts of the continent. However, the mechanisms behind its successful survival across arid and semi-arid African environments are relatively unknown. Here, we investigated the water balance of *S. frugiperda* across its developmental stages. Given the relationships between desiccation stress, temperature stress and other life history traits in arid ecosystems, we also measured interaction effects across metrics of these traits. Specifically, we measured basal body water content (BWC), water loss rates (WLRs) and the effects of desiccation pre-treatment on critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}) and fecundity. Body water content and WLR increased with age across larval instars. However, the effects of desiccation environments on WLRs were more dramatic for 5th and 6th larval instars. The 5th and 6th instars exhibited highest BWC and magnitude of WLRs plastic responses following desiccation treatment. The effects of desiccation pre-treatment on temperature tolerance were less apparent, only significantly improving CT_{min} in 2nd and 3rd larval instars and reducing CT_{max} in 5th instars. In addition, desiccation pre-treatment showed no significant effects on fecundity. These results show that water balance traits differ with developmental stage, while the effects of desiccation pre-treatment were more dramatic and inconclusive. The differential desiccation resistance, high propor-

tional BWC and no desiccation pre-treatment effects on fecundity may help the species survive in arid and semi-arid environments. This information provides insights into understanding *S. frugiperda* survival under desiccating arid and semi-arid tropical environments and is significant in predicting pest outbreaks.

Keywords

Desiccation pre-treatment, fall armyworm, fecundity, thermal plasticity, water balance

Introduction

In natural and managed ecosystems, insects encounter multiple overlapping environmental stressors which may affect their physiological fitness and survival (Gotcha et al. 2018; Mutamiswa et al. 2018, 2021). For example, there is a higher likelihood of increasing magnitudes and frequency of heat and desiccation stress as well as drought (associated with poor rainfall) as climate change ensues (IPCC 2014). As such, the interplay between heat stress and water deficit may exert physiological stress amongst insects and across ontogeny, and in all likelihood affecting organismal fitness, function and survival (Bubliy et al. 2012; Mitchell et al. 2017). Given that rapid dehydration occurs under dry environmental conditions, prolonged exposure to these conditions may negatively influence insect water balance and homeostasis (Guedes et al. 2015). Hence, survival may be highly dependent on body water content (BWC) management and desiccation tolerance. Various studies suggest that insects from tropical and drier environments have inherent adaptive water balance mechanisms such as reduction in cuticular permeability, reduced water loss rates (WLRs) through excretion and differential cuticular lipids composition and plasticity thereof (Hadley 1994; Gibbs et al. 1997; Gibbs 2002; Hoffmann et al. 2003a; Kellermann et al. 2009). Most empirical studies looking into the effects of insect responses to climate change have been skewed towards high temperature responses (Huey and Kearney 2020). However, precipitation and relative humidity (RH) fluctuations also exert significant stress to insects and tolerance to these stressors can largely affect species ecology (Chown et al. 2011; Kellermann et al. 2018). Moreover, increasing heat stress with climate change (see Stillman 2019; Xu et al. 2020) also comes with simultaneous high desiccation stress. Therefore, understanding water balance and heat stress, and the interactive responses of the two related stressors may be significant for survival in arid and semi-arid habitats and may be key determinants to invasion success for pest insect species (Kellermann et al. 2018).

Water balance is the ability of an organism to maintain constant body water levels under different environments (Yoder et al. 2003). It plays a pivotal role in determining insect diurnal and seasonal activity patterns, population dynamics and biogeography (Kleynhans and Terblanche 2011; Guedes et al. 2015). The ability of an insect to maintain water balance (water gain and loss equilibrium) is highly dependent on the moisture content of its immediate environment (Guedes et al. 2015). Insects lose water through the exoskeleton, evaporation through spiracles and tracheal system, oral or anal secretions, and excretion (Chown et al. 2011). However, the bulk of the water is

lost through the exoskeleton (Chown and Nicolson 2004; Rolandi et al. 2014). Given their small size, low fat-storage and a large surface area to volume ratio, maintenance of water balance in insects is a challenge, and significantly so in arid to semi-arid tropical changing environments (Gibbs et al. 1997; Gibbs et al. 2003; Chown et al. 2011; Weldon et al. 2013; Rolandi et al. 2014; Bujan et al. 2016). Insect water loss is also highly complex and varies with many factors including clines, species, environmental history (or plasticity) and ontogeny. However, the ontogenetic effects on water balance studies remain limited despite the significance of the traits for insects' population dynamics and biogeography. In contrast, insects gain water directly through drinking free water, indirectly through food and nectar as well as absorbing atmospheric water (Chown and Nicolson 2004; Guedes et al. 2015). As a result, the maintenance of hygric physiological homeostasis may be a key trait for survival and coupled with heat stress resistance, the two traits may be critical for surviving high temperature and desiccation in arid environments, typified with climate change. However, despite the significance of these two overlapping stressors, their interactive effects across ontogeny remain unknown for *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae), despite its significance for the pest species ecology.

Desiccation stress, commonly associated with xeric arid environments is one of the primary stressors influencing the distribution and behavior of insects in the tropics. As insects move from moister to drier environments, there is a high likelihood of experiencing increasingly desiccating conditions (Weldon et al. 2016). As a result, some organisms are spatially restricted to areas of optimal RH, or behaviorally track optimal RH conditions. For example, *Drosophila birchii* is usually restricted to rainforests due to its inability to tolerate desiccation stress (Hoffmann et al. 2003b; Bazinet et al. 2010). Hence, desiccation resistance (the ability of insects to withstand water loss) is fundamental in managing stress under rapidly shifting environments and can largely predict the fundamental niche of invasive pest species (IPCC 2014; Bujan et al. 2016). Desiccation resistance varies across geographic clines, body mass and biochemical composition (Guedes et al. 2015; Bujan et al. 2016), size and sex (Tejeda et al. 2014), age (Weldon and Taylor 2010), life stage and species (Weldon et al. 2013, 2016; Bujan et al. 2016). Similarly, within species desiccation resistance also varies across individual populations and development (Weldon and Taylor 2010). Insects have evolved some behavioral, morphological, physiological and biochemical mechanisms to withstand dehydration, and these are primarily hinged on increasing water storage, restricting WLRs and increasing tolerance to water loss (Gibbs et al. 2003; Chown and Nicolson 2004; Chown et al. 2011; Weldon et al. 2013, 2016). Behaviorally, insects can migrate to cooler moister microhabitats, aggregate and burrow to reduce desiccation stress (Guedes et al. 2015). In addition, insects can physiologically compensate for desiccation stress by converting stored fat to metabolic water, increasing cryoprotectants levels, decreasing metabolic rate and increasing reabsorption of water in the rectum (Benoit et al. 2007; Lopez-Martinez et al. 2009; Harrison et al. 2012). Using *Anastrepha ludens* (Tejeda et al. 2014) and Panama ant species (Bujan et al. 2016), previous studies showed a significant correlation between desiccation resistance and body size

owing to an increased BWC with body size. However, in *Bactrocera tryoni* (Weldon and Taylor 2010), desiccation resistance decreased with age and body size while for *Belgica antarctica*, the trait also varied across developmental stages (Benoit et al. 2007). This suggests complexity in determinants for desiccation stress resistance and warrants more investigations across different taxa before general conclusions can be made on desiccation resistance in insects.

Acclimation is a medium to long term, often reversible, change in phenotype in response to chronic exposure to sub-lethal stress under controlled conditions and within a single generation of an organism (Whitman and Agrawal 2009; Sgrò et al. 2016). This mechanism gives insects a performance advantage on a subsequent exposure to stress in the same environment. For example, insects exposed to prior stressful but sublethal dry environments tend to have lower WLRs on subsequent exposure to more severe desiccation stress (Terblanche and Kleynhans 2009; Chown et al. 2011). Various studies have pointed to the significant role desiccation acclimation plays in fitness and survival of different insect taxa, singly and interactively with other stressors e.g. temperature. For example, desiccation acclimation improved critical thermal minimum (CT_{min}) in larvae of *Chilo partellus* (Swinhoe), *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Mutamiswa et al. 2018). In addition, desiccation acclimation also increased heat tolerance (CT_{max}) in *Ceratitis rosa* Karsch adults (Gotcha et al. 2018) as well as larvae and adults of *Prostephanus truncatus* (Horn) (Mutamiswa et al. 2021). These studies suggest potential positive interactive effects across divergent stress traits, a phenomenon that may be key to maintaining fitness under stressful heterogeneous environments (see Sinclair et al. 2013).

Spodoptera frugiperda is a tropical insect of American origin which was first reported in Africa in 2016 (Goergen et al. 2016; Stokstad 2017). Since then, it has successfully invaded African countries (Stokstad 2017), Asia (FAO 2018a; Sharanabasappa et al. 2018; Yigezu and Wakgari 2020) and recently Australia (Maino et al. 2021). It is highly polyphagous (Cock et al. 2017; Montezano et al. 2018; Tambo et al. 2019) but it mainly prefers maize (Goergen et al. 2016; Abrahams et al. 2017; FAO 2018b). Its resilience and ability to survive all year round in African countries has been a topic of great concern (Early et al. 2018; Megersa and Tamiru 2019; Keosentse et al. 2021). Previous studies have assayed its thermal tolerance (Barfield et al. 1978; Du Plessis et al. 2020; Keosentse et al. 2021) and developed models to predict its spatio-temporal distribution (Westbrook et al. 2016; Early et al. 2018; Du Plessis et al. 2018). However, mechanisms behind its invasion success in tropical and arid environments remain poorly elucidated. The movement of *S. frugiperda* across humid and dry African environments, its ingression and naturalization may be better explained by efficient mechanisms of water balance, heat tolerance and their interactive effects thereof. Empirical studies on water balance have been reported in *Sitophilus zeamais* Motschulsky (Guedes et al. 2015), *Ceratitis capitata* (Wiedemann), *Ceratitis cosyra* (Walker) and *C. rosa* (Weldon et al. 2016). However, no studies have investigated developmental stage related differences in water balance traits of *S. frugiperda*. In addition, no studies have looked at the interaction of desiccation and high temperature stress for the

species, despite being significant for fitness in dry tropical habitats. Against this background, we investigated developmental stage related differences in water balance in *S. frugiperda*. Furthermore, we also tested the effects of desiccation environments on the physiological traits *vis* WLRs and thermal tolerance and ecological traits, including effects on fecundity. Given *S. frugiperda*'s high invasion propensity under shifting environments, we hypothesize that desiccation pre-treatment capacity coupled with highly integral water balance may enhance its fitness in novel arid and semi-arid environments. We also hypothesize that there is variation in BWC, WLRs and thermal tolerance following acclimation across *S. frugiperda* developmental stages. The information generated here may help in modeling species distribution and developing management options under globally shifting environments.

Materials and methods

Insect culture and rearing conditions

Wild populations of *S. frugiperda* 1st instar neonate larvae from different cohorts of parents were collected from maize fields in Bobonong village (22.26195°S, 28.99985°E) in the Central district of Botswana during austral summer (November 2020 to March 2021). They were mass reared in the laboratory on artificial diet (see Tefera et al. 2019) under optimum conditions i.e. 28 ± 1 °C; $65 \pm 5\%$ RH; 12:12 light: dark photoperiod in climate controlled chambers (HPP 260; Memmert GmbH + Co.KG, Germany). Since cannibalism is predominant amongst late larval instars (Chapman et al. 1999), each late instar larva (3rd onwards) was individually placed in a separate 100 ml plastic vial with perforated screw-cap lid and artificial diet until pupation. Pupae were maintained in open Petri dishes in Bugdorm rearing cages (320 cm³; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) until adult eclosion. Following eclosion, adults were provided with 25% sugar water-soaked cotton wool in a 60 ml plastic vial for food *ad libitum*. At least two potted maize plants (2 weeks old) were placed in each rearing cage as oviposition substrate for gravid females. After hatching the 1st instar larvae were transferred to artificial diet for subsequent rearing. For all experiments, all larval instars, 24–48 h old pupae and adults (unsexed) F₁ populations were used. Larval instar stages were demarcated using head capsule size (see Montezano et al. 2019).

Body water- and proportional body water-content

Twenty insects from each larval instar (1–4) were individually placed in 0.5 ml microcentrifuge tubes of known weight while instars (5–6), pupae and moths were individually placed in 2 ml microcentrifuge tubes of known weight. The initial mass of each insect was measured (to 0.0001 g) on an analytical balance (AS220.R2, RADWAG, Poland). Thereafter, the insects were placed in a Memmert drying oven (UF160, Memmert, Germany) set at 60 °C for 48 hours. Dry mass was measured (to 0.0001 g) on a

microbalance after allowing insects to cool under laboratory temperature (28 °C) for 15 minutes. Dry mass was subtracted from initial mass to determine initial BWC (see complete methods in Bazinet et al. 2010; Weldon et al. 2018). Proportional BWC, basal body water (g) divided by wet mass (g) was calculated and used for analyzing developmental stage effects for this trait.

Water loss rates

Larval instars, pupae and adults (N = 20 for each developmental stage) were individually placed in pre-weighed perforated 0.5 ml and 2 ml microcentrifuge tubes of known weight. The weight of each insect-carrying tube was later measured on an analytical microbalance. Thereafter, insect weight was determined by subtracting initial weight of tube from weight of insect-loaded tube. These tubes were placed on top of wire gauze in a glass sealed desiccator containing 80 g silica gel (equivalent to 7% RH) in climate chambers set at 28 °C; 65% RH for treatment while controls were placed in a desiccator without silica gel. After every 12 h, the insects were removed from each climate chamber, weighed in their tubes to calculate weight loss, and then immediately placed back in the chambers for subsequent recordings. This was repeated for 48 hours for all the developmental stages. Water loss rate (mg hr⁻¹) over the 48 h experimental duration was calculated as follows:

$$WLR = \frac{M_1 - M_2}{T}$$

where WLR = water loss rate, M_1 = initial body mass, M_2 = final body mass and T = time (hours) following methods by Weldon et al. (2016) and Mutamiswa et al. (2021).

Effects of desiccation pre-treatment on physiological (critical thermal limits [CTLs]) and ecological traits (adult fecundity)

Desiccation pre-treatment assays were carried out using standard established protocols (see Bauerfeind et al. 2014; Mutamiswa et al. 2018) before measuring physiological and ecological traits. *Spodoptera frugiperda* larval instars and adults were individually placed in ten perforated 0.5 ml and 2 ml microcentrifuge tubes. The tubes were placed on top of wire gauze in a glass sealed desiccator containing 80 g silica gel. Thereafter, the desiccator was placed in a climate chamber under optimum conditions for 24 h. Thermocron iButtons (Model DS1920; Dallas Semiconductors, Dallas, Texas) were used to measure temperature and RH inside the desiccator at 30-min intervals. After 24 h, the insects were transferred to artificial diet for a further 12 h under optimum conditions before measuring CTLs and fecundity. Control organisms were maintained in climate chambers at (28 °C; 65% RH) before measuring their CTLs and fecundity.

Critical thermal limits

Ten individual *S. frugiperda* larvae (of each 1–6th instars) and adults (mixed sex), from desiccation pre-treatment were placed into a double jacketed chamber ('organ pipes')

connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to allow for subzero temperatures. A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the middle/control test tube to record chamber temperature. Both critical thermal- maxima (CT_{max}) and -minima (CT_{min}) experiments commenced at a set point temperature of 28 °C (with 10 minutes equilibration time) from which temperature was ramped up (CT_{max}) and down (CT_{min}) at a rate of 0.25 °C/min until their CTLs were reached. The experimental procedure was repeated twice for each trait and treatment (1–6th instars plus adults) to yield sample sizes of $N = 20$ for each treatment. Controls were maintained in climate chambers under optimum conditions (28 °C; 65% RH) before measuring their CTLs. Critical thermal limits were defined as the lower (CT_{min}) and upper (CT_{max}) temperatures at which each individual insect lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding) (see discussions in Nyamukondiwa and Terblanche 2010; Mutamiswa et al. 2017).

Fecundity

Twenty replicate pairs of treatment (desiccation pre-treated) 1–2 day old female and male adults of *S. frugiperda* were placed in separate rearing cages in climate chambers under optimum conditions. Each pair was provided with a cotton wad soaked in 25% sugar-water and a potted maize plant (2 weeks old) for oviposition. Control adult pairs (male and female) were maintained at optimal conditions (28 °C; 65% RH) until egg laying. Insects were allowed to mate, oviposit and removed from the experimental cages on day 8. Eggs were allowed to hatch on maize plants and emerging 1st instar neonate larvae were counted and removed from the cage immediately after hatching. Fecundity was defined as the number of emerging 1st instar neonate larvae per pair following adult oviposition for the 7 days duration.

Data analyses

Data analyses were carried out in STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma) and R version 3.3.0 (R development Core Team 2020). Data were first checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests respectively. For BWC, proportional BWC, fecundity and CTLs data, linear model assumptions of constant variance and normal errors were met while WLR data failed to conform to assumptions of analysis of variance (ANOVA). Therefore, BWC, proportional BWC and fecundity results were analyzed using one-way ANOVA while CTLs results were analyzed using full factorial ANOVA with developmental stage and treatment being the categorical factors whereas BWC, proportional BWC, fecundity, CT_{min} and CT_{max} were the dependent variables. Water loss rates were analyzed using generalized linear model (GLM) assuming a Gaussian distribution and an identity link function in R with duration,

developmental stage and time being the categorical factors and WLR being the dependent variable. Tukey-Kramer’s *post-hoc* tests were used to separate statistically heterogeneous groups.

Results

Body water content

Body water content varied significantly across developmental stages ($P < 0.001$) (Table 1, Fig. 1A). The mean BWC for all larval instars (1st–6th), pupae and adults were 0.01 ± 0.001 g, 0.03 ± 0.002 g, 0.06 ± 0.002 g, 0.11 ± 0.005 g, 0.19 ± 0.007 g, 0.29 ± 0.01 g, 0.15 ± 0.005 g and 0.05 ± 0.002 g respectively (Fig. 1A). Body water content significantly increased with larval instar level with instar 1 recording the lowest BWC whilst the 6th instar recorded the highest BWC amongst all tested developmental stages (Fig. 1A). However, there was no significant difference in BWC between 1st and 2nd, 2nd and 3rd and across 2nd and 3rd larval instars and that of adults (see Fig. 1A). Proportional BWC also differed significantly among developmental stages ($P < 0.001$) (Table 1, Fig. 1B). Adults recorded the lowest proportional BWC (0.63 ± 0.01), followed by pupae (0.71 ± 0.005) while larvae recorded the highest proportional BWC, albeit it was not significantly different amongst all instars (Fig. 1B).

Water loss rate

Generally, WLRs seemed to increase with larval instar, consistent with the constituent BWC. Water loss rate significantly varied across developmental stages, and time ($P < 0.001$) and not significantly among treatments ($P > 0.01$) (Table 2, Fig. 2). For all tested developmental stages, there seemed to be no significant effect of desiccation environment on WLRs except for 5th and 6th larval instars (see Fig. 2), developmental stages that also recorded the highest BWC (Fig. 1A). These trends were consistent across the different times (12, 24, 36 and 48 h). Moreover, WLRs gradually decreased over time for instars 4, 5 and 6 as well as adults of *S. frugiperda*. First instar larvae recorded the lowest WLR while 6th instar larvae recorded the highest WLR across time (Fig. 2). The only significant interaction ef-

Table 1. Summary statistical results from a one-way ANOVA showing the effects of developmental stage on BWC and proportional BWC in *S. frugiperda*. SS = sums of squares, DF=degrees of freedom, BWC=body water content.

Trait	Effect	SS	DF	MS	F	P
BWC	Intercept	1.99	1	1.99	3115.48	< 0.001
	Developmental stage	1.25	7	0.18	278.72	< 0.001
Proportional BWC	Intercept	99.48	1	99.48	51920.61	< 0.001
	Developmental stage	0.85	7	0.12	63.45	< 0.001
	Error	0.29	152	0.00192		

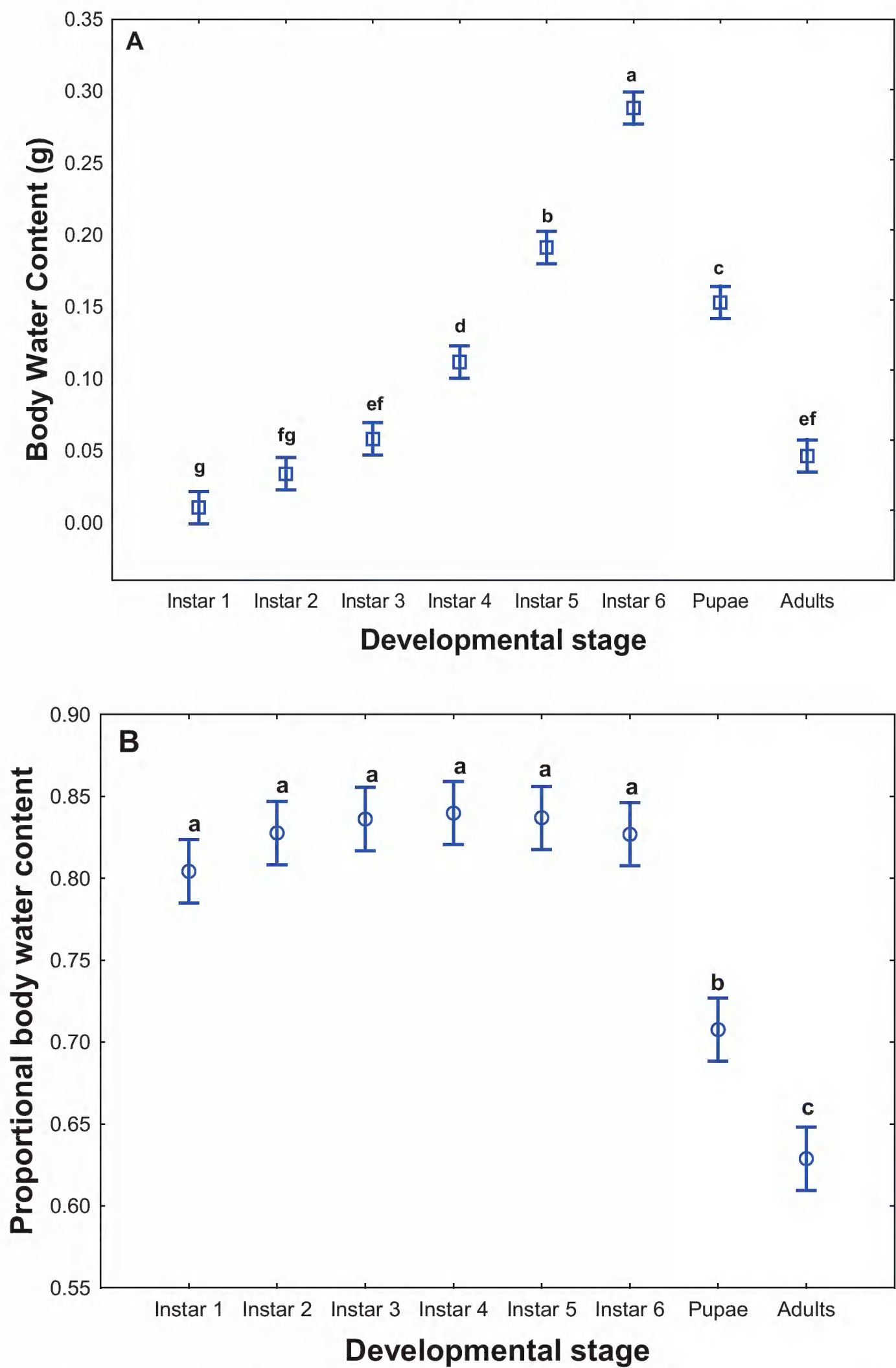


Figure 1. *Spodoptera frugiperda*'s body water content **A** and the proportional body water content **B** for larvae (all six instars), pupae and adults (48 h old). Error bars represent 95% confidence limits (N = 20). Means with the same letter are not significantly different from each other.

Table 2. Summary statistical results from factorial analysis using generalized linear model (GLM) on the effects of desiccation, developmental stage and interaction thereof on *S. frugiperda* water loss rates. All analyses were done in R version 3.3.0. DF = degrees of freedom.

Effect	DF	χ^2	P
Desiccation	1	0.05	0.65
Developmental stage	7	765.66	<0.001
Time	3	10.51	<0.001
Desiccation *Developmental stage	7	52.19	<0.001
Desiccation *Time	3	0.54	0.546
Developmental stage*Time	21	8.83	0.033
Desiccation *Developmental stage*Time	21	2.42	0.985

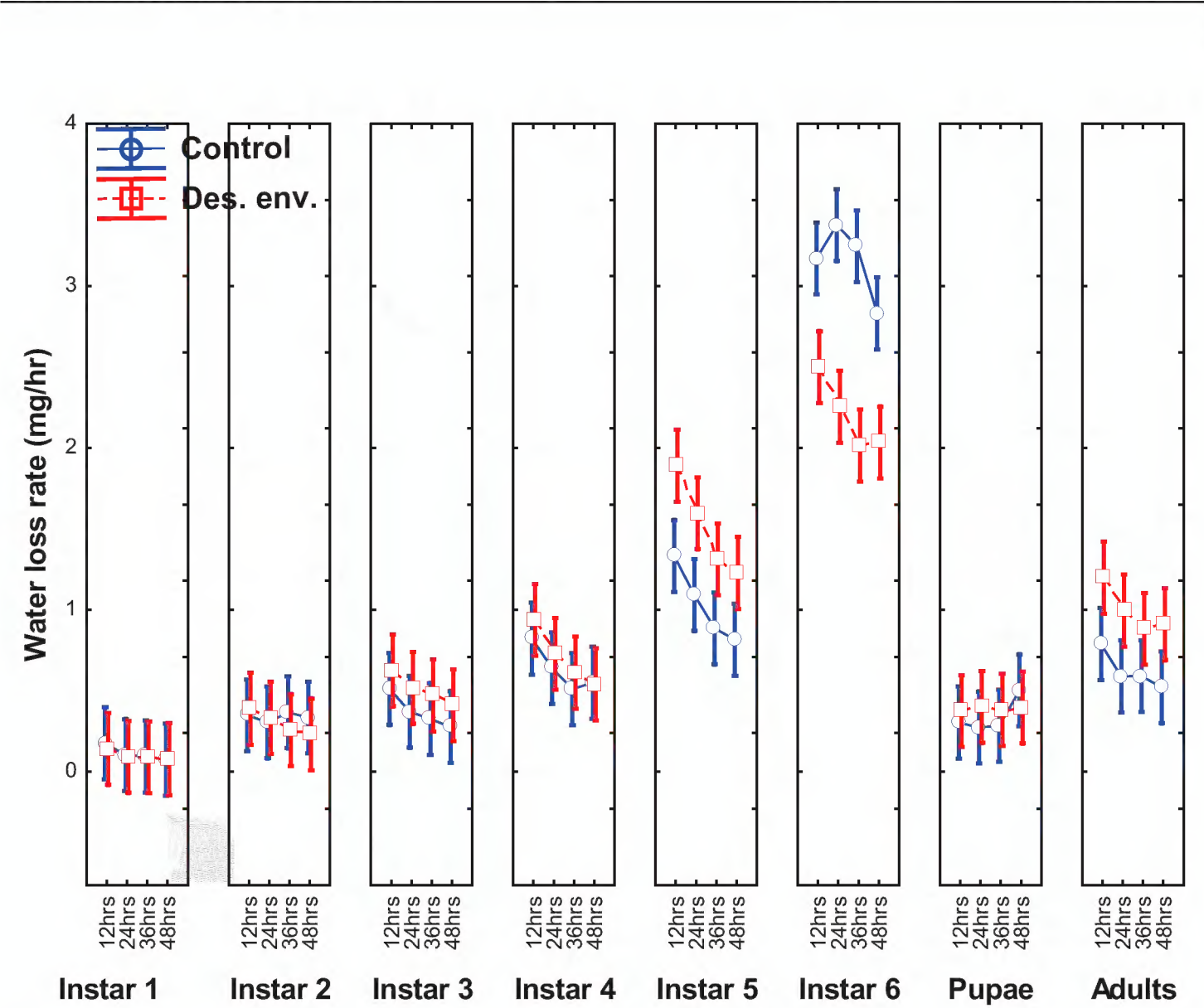


Figure 2. Water loss rates of *S. frugiperda* larvae (instars 1–6), pupae and adults following 12, 24, 36 and 48 h desiccation exposure at 7% RH. Error bars represent 95% confidence limits (N = 20 per treatment group). Des. env. = desiccation treatment (7% RH).

fect was found between treatment and developmental stage (Table 2). No mortalities were recorded for all individuals in each developmental stage during the WLR experiments.

Effects of desiccation pre-treatment on CTLs and fecundity

Following desiccation pre-treatment, all individuals and across all developmental stages survived acclimation stress before subsequent CTLs and fecundity experiments. Desic-

Table 3. Summary statistical results from a factorial ANOVA (CT_{min} and CT_{max}) and one-way ANOVA (fecundity) showing effects of desiccation pre-treatment, developmental stage and interaction thereof on *S. frugiperda* critical thermal limits (CT_{min} , CT_{max}) (larvae and adults) and adult fecundity, measured as number of 1st instar neonates larvae produced during a 7 days incubation period. SS = sums of squares, DF = degrees of freedom, CT_{min} = critical thermal minimum, CT_{max} = critical thermal maximum.

Trait Effect	SS	DF	MS	F	P
CT_{min} Intercept	6796.501	1	6796.501	27892.11	<0.001
Developmental stage	455.48	6	75.914	311.54	<0.001
Desiccation pre-treatment	0.350	1	0.350	1.44	0.232
Developmental stage* desiccation pre-treatment	23.001	6	3.834	15.73	<0.001
Error	64.817	266	0.244		
CT_{max} Intercept	655463.3	1	655463.3	58937.37	<0.001
Developmental stage	290.5	6	48.4	4.35	<0.001
Desiccation pre-treatment	47.7	1	47.7	4.29	0.039265
Developmental stage * Desiccation pre-treatment	155.1	6	25.8	2.32	0.033290
Error	2958.3	266	11.1		
Fecundity Intercept	5995405	1	5995405	852.7159	<0.001
Desiccation pre-treatment	3497	1	3497	0.4974	0.485
Error	267176	38	7031		

cation pre-treatment effects were generally dramatic for CT_{min} , CT_{max} and across tested developmental stages ($P > 0.01$) (Table 3). For example, desiccation pre-treatment impaired cold tolerance (higher CT_{min} temperature) in 1st instar larvae while improving the same trait (lower CT_{min} temperature) in 2nd and 3rd instar larvae (Fig. 3A). Similarly, desiccation pre-treatment impaired heat tolerance (lower CT_{max} temperature) in 5th larval instars while it appeared to have no significant effect on all other treated developmental stages (Fig. 3B). Both controls and desiccation pre-treated adults recorded significantly lowest CT_{min} (highest cold tolerance) while the rest of the treated developmental stages appeared to have CT_{min} ranging between 4.7 and 6.5 °C (see Fig. 3A). A significant interaction effect in CT_{min} was recorded between developmental stage and desiccation pre-treatment ($P < 0.001$) (Table 3). Similar to CT_{min} , the interaction effects of developmental stage × desiccation pre-treatment were significant for CT_{max} ($P < 0.05$) (Table 3).

Desiccation pre-treatment did not significantly influence fecundity ($P > 0.05$). The number of 1st instar neonates recorded in controls versus desiccation pre-treatments was 377.8 ± 10.43 and 396.5 ± 6.87 respectively (Table 3, Fig. 3C).

Discussion

One of the key environmental stresses insects face in both natural and agroecosystems is dehydration (Gibbs et al. 1997). Given that the incidences and severity of droughts and high temperature stress will increase with climate change in tropical regions, the population persistence of insects may be hinged on adaptation to these two stresses, either through high basal tolerance or integrated stress resistance (Guedes et al. 2015; Bujan et al. 2016). While Botswana is arid to semi-arid, microclimatic conditions experienced

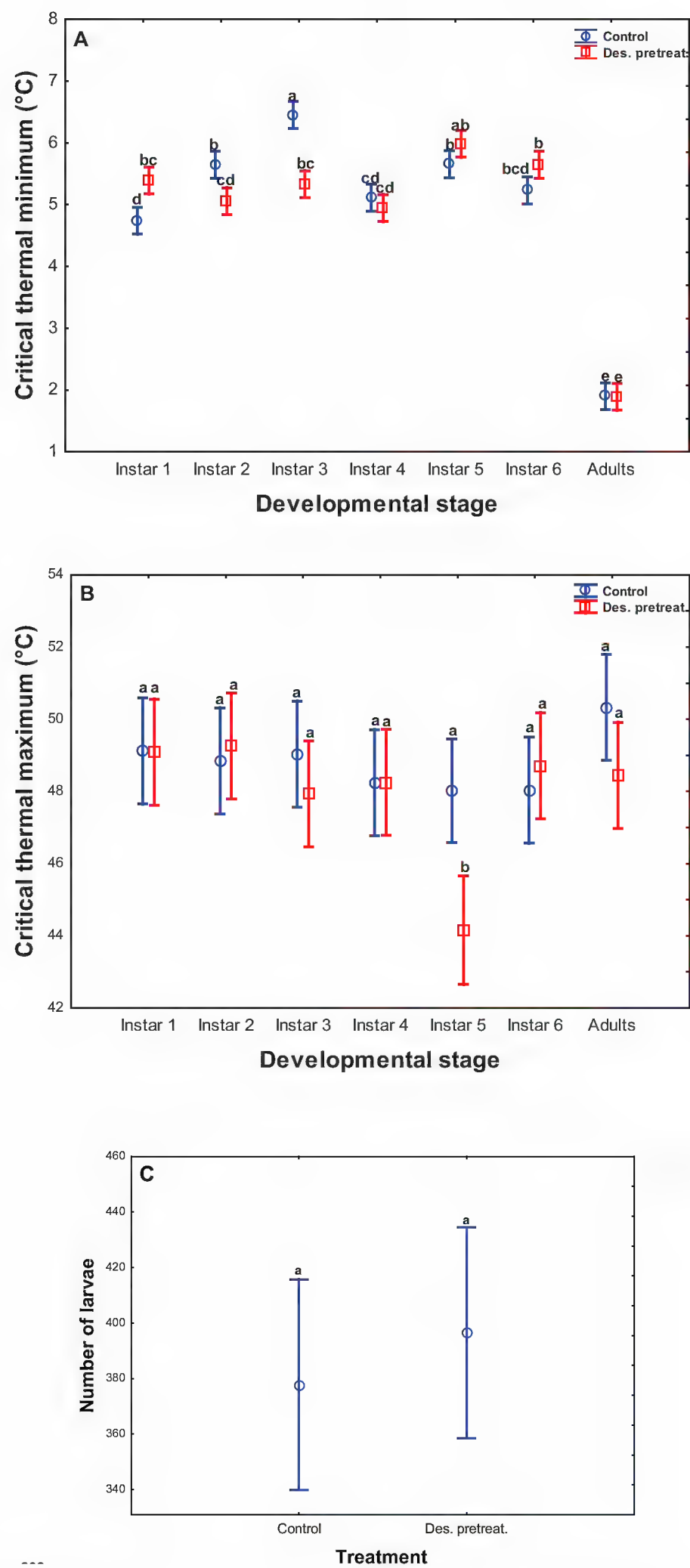


Figure 3. Effects of desiccation pre-treatment (des. pretreat.) on critical thermal minimum **A** critical thermal maximum **B** for *S. frugiperda* larvae (all six instars) and adults and **C** fecundity of *S. frugiperda* adults. Error bars represent 95% confidence limits (N = 20) and means with the same letter are not significantly different from each other.

locally show that *S. frugiperda* life stages likely experience episodes of high temperatures and desiccation, associated with heat waves and droughts during recent austral summers (see Keosentse et al. 2021). This indicates frequent exposure to overlapping acute and chronic environmental stresses, such as heat and desiccation. Our results show differential levels of water balance traits (basal BWC, proportional BWC and WLRs following desiccation) across developmental stages. Furthermore, our results also show dramatic effects of desiccation pre-treatment on physiological traits (CTLs), but without apparent structuration while effects of desiccation pre-treatment on fecundity were not apparent.

Body water content increased with larval instar age, with 1st and 6th larval instars recording the lowest and highest BWC respectively. The result is in keeping with Tarusikirwa et al. (2021) who recently reported similar trend in a related lepidoptera, *Tuta absoluta*, with larval instars' BWC increasing with age. In addition, this result is also consistent with findings by Bujan et al. (2016) that showed BWC increases with age and body size, likely extending the significant role of body size on BWC. Furthermore, body water reserves may be limited in smaller relative to larger developmental stages owing to higher surface area to body mass ratio (Krupp et al. 2020). This characteristic also makes smaller developmental stages more vulnerable to water loss than larger ones. This result suggests that early larval neonates may be more vulnerable to desiccating environments than the later stages that have higher BWC and relatively lower surface area to volume ratio. Bujan et al. (2016) reported higher BWC in canopy ants (larger insects) than understory ants (smaller insects). A comparison of 4th–6th instar larvae and adults showed that late larval instars had higher BWC than adults in keeping with Tarusikirwa et al. (2021) who reported higher BWC in *T. absoluta* 4th instars than adults. This may be attributed to their diets and habitat environments. Larvae feed on plant parts, full of sap that may provide higher amounts of water. Furthermore, adult *S. frugiperda* may be more 'terrestrial' than larval stages; the latter are always within microhabitats of the host plants, where they are protected from desiccation. This may partly account for the lower BWC of adults relative to the late instar larvae. This finding is congruent with Yoder et al. (2003) who reported higher BWC in *Stethorus nigripes* larvae than adults. In addition, pupae had higher basal BWC than adults. Indeed, from an evolutionary perspective, most 'immobile' developmental stages (e.g. pupae) always compensate for stress resistance through exhibiting inherent basal higher stress resistance than more mobile (e.g. adults) stages that can behaviorally compensate (Chown and Nicolson 2004). This partly accounts for the higher basal BWC of the pupae relative to adults reported here. Benoit et al. (2007) nevertheless reported higher BWC in 3rd versus 4th larval instars of *Belgica antarctica* suggesting ambivalent effects on the effects of developmental stage and body mass on basal BWC. Nevertheless, adults recorded lower proportional BWC than larval instars, in contrast to Tarusikirwa et al. (2021) who reported higher proportional BWC in *T. absoluta* adults relative to larvae. This suggests that proportional BWC in insects may be species and developmental stage dependent. We speculate here that a lower proportional BWC for adults may mean that adult *S. frugiperda* may use other mechanisms for avoiding desiccation. For example, adults are nocturnal (are mostly active during humid evenings) (Rojas et al. 2004) and

hide under host leaves during desiccating daytime environments (Rwomushana 2019). This may mean that behavioral adaptation may be more important for desiccation tolerance in adults relative to other developmental stages.

Water loss rates increased with age amongst larval instars, consistent with the increase in BWC with age across larvae. First and 6th larval instars recorded the lowest and highest WLRs respectively and across the recorded times (12–48 h). These results are closely linked with BWC amongst larval instars since it also increased with larval instar age. This result is contrary to Benoit et al. (2007) who reported higher WLRs in 3rd than 4th larval instars of *B. antarctica*. Dehydration resistant insects are usually associated with higher lipid content or a heavily waterproofed cuticle that reduces water loss (Benoit et al. 2007). The variation in WLRs reported here may be linked to differential body lipid content among larval instars. Furthermore, significant differences in WLRs were only evident for 5th and 6th instar larvae. This may be because of their basal higher BWC which may allow desiccation pre-treatment responses to be evident as compared to early instars, whose relatively lower BWC may result in desiccation pre-treatment having little effects on plasticity of WLR. High WLRs recorded in the controls in instar 6 could be a combination of dehydration and mass loss due to consumption of reserves for energy since insects were not fed during the experiment. Decreased WLRs in the 6th instar following desiccation pre-treatment may also be due to the stress induced sit and wait strategy (Lighton and Fielden 1995). When exposed to stress, insects may reduce their metabolic activity hence reducing energy consumption and lowering water loss through gaseous exchange due to decreased respiration (Lighton and Fielden 1995). However, desiccation pre-treatment led to an increase in WLR in the 5th instar larvae, albeit not significant. Nevertheless, a gradual decrease in WLRs over time in 4th, 5th and 6th instars as well as adults relative to other developmental stages indicate that water balance may be life stage or cuticular permeability dependent, hence suggesting the role of these developmental stages (4th, 5th, 6th instars and adults) in surviving high temperature and desiccating arid African environments. Differential WLRs (low overall magnitude) recorded here following exposure to desiccating environments may highlight enhanced water conservation under constant organismal activity levels. Furthermore, it may also highlight potential organismal reduced metabolic activity during periods of stress, reflecting onto lower energy consumption as well as lower water loss through gaseous exchanges' due to decreased respiration (Benoit et al. 2007). While cuticular permeability was not measured across life stages in the current study, this warrants further investigation to elucidate these differential responses amongst life stages. Although pupae recorded higher BWC than adults, interestingly their WLRs were lower than those of adults. This supports the notion that immobile stages have developed inherent survival mechanisms given their inability to behaviorally thermoregulate under stressful desiccating environments. Pupae have a hard shell/cocoon that prevents abrasion of surface water-proofing layer of the cuticle hence improving desiccation resistance (reviewed in Danks 2000).

Our results showed improved cold tolerance (lower CT_{min}) for 2nd and 3rd larval instars following desiccation pre-treatment indicating some cross tolerance effects.

This is in consonance with Mutamiswa et al. (2018), who reported an enhanced CT_{min} in related species, *C. partellus*, *B. fusca* and *S. calamistis* larvae following desiccation pre-treatment. In addition, our results are in keeping with Bayley et al. (2001) who reported improved cold tolerance following desiccation stress in soil collembolan, *Folsomia candida*. These plastic responses in 2nd and 3rd larval instars indicate fitness and survival edge relative to other larval instars under desiccating environments. Although adults did not show any plastic responses following desiccation pre-treatment, they recorded the lowest CT_{min} indicating highest cold tolerance amongst all tested developmental stages outside acclimation responses. While insects have been reported to upregulate heat shock proteins (Hsps) synthesis in response to desiccation stress (e.g. Feder and Hofmann 1999), Hsps also improve cold tolerance (Chown and Nicolson 2004). Thus, this potential overlap in resistance mechanisms, albeit not measured here, may account for this integrated stress resistance in our results. Furthermore, desiccation pre-treatment impaired cold tolerance (higher CT_{min}) than controls, while it had no effects on all other measured parameters. Impairment of cold tolerance following desiccation pre-treatment suggests a cost of desiccation on cold tolerance, which may be developmental stage dependent. These fitness costs may be caused by the cumulative effects of both the sub-lethal desiccation pre-treatment and the cold tolerance traits that negatively affect fitness traits. Coupled with no acclimation effects of all other treatments for CT_{max} , this may point to a cross-susceptibility to temperature (especially heat) and desiccation across *S. frugiperda* development. Similar trends have been reported in *Sarcophaga crassipalpis* (Tammarielo et al. 1999) as well as *S. calamistis* and *C. partellus* larvae (Mutamiswa et al. 2018). Given these differential CTLs following desiccation pre-treatment in the current study, this suggests that physiological stress responses are life stage and trait dependent in *S. frugiperda* and that plastic interactive effects of desiccation and temperature may not yield significant beneficial acclimation responses.

Desiccation pre-treatment did not significantly affect fecundity in *S. frugiperda*. This suggests that invasive *S. frugiperda* still remains competitively fecund under highly desiccating arid environments and this trait may highly contribute to its rapid establishment in dry and hot tropical environments. The results are in agreement with Tajeda et al. (2016) who reported a similar trend between desiccation acclimated and control *A. ludens*. However, our results are in contrast with Benoit et al. (2010) who reported significantly lower fecundity in aquatic *Culex pipiens* following multiple bouts of dehydration. Nevertheless, the lack of dehydration effects on fecundity reported here may be a key determinant for increasing propagules of *S. frugiperda* when introduced in novel, arid and stressful habitats. Our fecundity assays were done following desiccation pre-treatment of adults only following acute acclimation pre-treatment. Given that more chronic acclimation treatments and/or carryover effects cascading into next life stages may alter adult fitness, future studies should focus on pre-treating earlier developmental stages under these aforementioned conditions to elucidate their effects on reproductive capacity of this invasive insect pest.

Conclusion

This work reports developmental stage differences in water balance and performance of *S. frugiperda* and implications on potential invasion under changing environments. Our results show that 1) basal BWC increased with age among larval instars and that this trend was also consistent with WLRs. However, proportional BWC seemed to decrease with developmental stage, and was lowest in adults. We also show that 2) WLRs increased with age in larval instars, and that pupae had lower WLRs than adults. However, the effects of desiccation environment on WLRs were beneficial for 6th relative to 5th instar larvae, suggesting the role of developmental stage on beneficial acclimation responses. Third, desiccation seemed to generally have more dramatic effects across development, suggesting complexity associated with acclimation responses that may be intertwined across development. Last, the effect of prior dehydration stress had no effects on fecundity, likely aiding the reproduction and fitness of this species in accumulating propagules under stressful arid environments. Physiological responses reported here may partly account for the thriving invasive populations of *S. frugiperda* in arid and semi-arid African habitats. Future studies should focus on understanding physiological mechanisms underlying water conservation in this invasive species. Similarly, more work may be needed to refine and make more conclusive plastic interactive effects of temperature and desiccation on *S. frugiperda* fitness traits. This information provides insights into understanding invasive species adaptation under desiccating environments and is significant in predicting spatio-temporal invasive pest outbreaks under changing abiotic environments.

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